Weed Science 2009 57:48–53

Distribution of Glyphosate-Resistant Horseweed (*Conyza canadensis*) and Relationship to Cropping Systems in the Central Valley of California

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Horseweed is an increasing problem in perennial crops and noncrop areas of the Central Valley of California. Similar to the situation in glyphosate-tolerant crops in other regions, glyphosate-based weed-management strategies in perennial crops and noncrop areas have resulted in selection of a glyphosate-resistant horseweed biotype in California. Research was conducted to determine the level of resistance to glyphosate in horseweed using an in vivo enzyme assay and to determine the distribution of the resistant horseweed biotype in central California. The resistant biotype was 4.8-fold more resistant to in vivo glyphosate exposure compared with the susceptible biotype, although enzyme function was inhibited in both biotypes at high glyphosate concentrations. An intermediate in vivo glyphosate dose was used to discriminate between glyphosate-resistant and glyphosate-susceptible individuals in a roadside survey conducted in 2006 to 2007. Overall, 62% of the individuals tested from the Central Valley were classified as resistant to glyphosate. Resistant individuals were found at most locations throughout the Central Valley, although the proportion of resistant individuals was slightly lower in the northern-most area. No correlation could be made between proportion of resistant or susceptible individuals and land use patterns likely because of long-distance seed dispersal or different selection pressure for resistant biotypes on field margins compared with that within fields. Horseweed with an economically significant level of resistance to glyphosate is already widely distributed in the Central Valley of California. Grower awareness of the problem and adoption of best management practices are needed to minimize the effects of horseweed in this highly productive and diverse agricultural region.
Nomenclature: Glyphosate; horseweed, *Conyza canadensis* (L.) Cronq. ERICA.
Key words: Glyphosate resistance, herbicide resistance, shikimate assay.

Horseweed is a common annual weed in North America. Because it is a prolific seed producer with windblown seeds, horseweed often is an early colonizer of field margins, roadsides, industrial areas, and other infrequently disturbed sites (Dauer et al. 2007). Horseweed does not tolerate soil disturbance well and is sensitive to several herbicides commonly used in large acreage crops and, thus, usually is not a major problem in conventional annual cropping systems in the United States. However, in recent years, many annual crop growers have switched to a reduced-tillage or no-tillage cropping system, which increases the reliance on herbicides for control of weeds like horseweed.

Evolution of resistance to weed control measures is largely a function of selection pressure; thus, reducing tillage operations and relying solely on herbicides for weed control increases the selection pressure for herbicide-resistant weed biotypes. Failure to adequately rotate among herbicide modes of action can add even more selection pressure for weeds resistant to the primary herbicide used in the cropping system (Heap and LeBaron 2001). In some crops in the United States, including soybean (*Glycine max* L. Merr.), corn (*Zea mays* L.), and cotton (*Gossypium hirsutum* L.), the introduction of glyphosate-resistant (''Roundup Ready'') cultivars has increased selection pressure for resistant weed biotypes because of repeated applications of glyphosate and has decreased the need for preplant tillage and in-season cultivation for weed control (Gianessi 2004; Nandula et al. 2005).

The phenotypic plasticity of the weed species also can affect the evolution of herbicide resistance (Heap and LeBaron, 2001). *Conyza* spp. appear to have a high propensity for evolution of herbicide resistance, and, in recent years, horseweed and hairy fleabane [*Conyza bonariensis* (L.) Cronq.] biotypes with resistance to several herbicide modes of action have been reported around the world (Heap 2008). Glyphosate-resistant (GR) horseweed was first reported in 2001 and has since been reported in five countries and 16 U.S. states (Heap 2008; Van Gessel 2001).

In recent years, horseweed has become more prevalent in orchards, vineyards, canal banks, roadsides, and fallow areas throughout much of the Central Valley of California. In this diverse cropping region, dominated by perennial crops, horseweed can reduce crop growth and productivity in newly planted orchards and vineyards, interfere with furrow and microjet irrigation efficiency, and interfere with pest control and harvest operations. Producers of perennial tree fruits, tree nuts, and grapes (*Vitus vinifera* L.) in the Central Valley commonly use tillage or mowing to control weeds between the crop rows but typically rely on herbicides for control of weeds within the crop row. Fruit and nut producers often use multiple herbicide applications or combinations of herbicides, such as paraquat, glyphosate, glufosinate, oryzalin, and oxyfluorfen, to control weeds within the row (CADPR 2008).

Although the trend for increased reliance on glyphosate and reports of GR weeds have been particularly obvious in glyphosate-tolerant crops (Nandula et al. 2005; Shaner 2000), orchard and vineyard operations in the Central Valley of California also commonly use this broad-spectrum, relatively inexpensive herbicide for weed control. For example, in 2006, glyphosate was applied at least once to 43% of all grapes (324,000 ha production), 43 to 66% of all stone fruit (*Prunus* spp L.) (89,000 ha production), and 47 to 61% of all almond [*Prunus dulcis* (Mill.) D. A. Webb var. *dulcis*], walnut (*Juglans regia* L.), and pistachio (*Pistacia vera* L.) acreage (364,000 ha production) in California (USDA-NASS 2006). Given the evolution of GR horseweed in annual cropping systems in other states and the increased reliance on glyphosate in California perennial cropping systems, it is not surprising that there have been several recent reports of GR weed biotypes in California, including horseweed, hairy fleabane, and Italian

DOI: 10.1614/WS-08-103.1
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ryegrass (*Lolium multiflorum* Lam.) (Jasnieniuk et al. 2008; Shrestha et al. 2007, 2008; Simarmata et al. 2003).

The evolution of GR *Conyza* spp. biotypes may, at least partly, explain the increased abundance of these weeds in the San Joaquin Valley, CA; however, very little data are available on the level of resistance or the distribution of the resistant biotype in the region. Although it has been suggested that the original infestations of the resistant horseweed in the southern Central Valley may have been selected in orchards or irrigation canal systems, no research has been conducted to determine whether these areas are the primary sites with the resistant biotypes. Therefore, the objectives of this research were to (1) determine the level of glyphosate resistance in horseweed collected in the Central Valley of California using an in vivo shikimate assay, (2) determine the distribution of the resistant biotype in the region using a shikimate assay as a rapid screening tool, and (3) determine whether the resistant biotypes are associated with particular cropping systems.

Materials and Methods

In Vivo Shikimate Accumulation Technique. Horseweed sensitivity to glyphosate was characterized using an in vivo assay detailed by Koger et al. (2005) and Shaner et al. (2005). In this assay, excised leaf tissue is exposed to glyphosate in an incubation solution, and the level of shikimic acid accumulation is used to determine the level of inhibition of the target enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Mueller et al. 2003). Briefly, five 4-mm leaf disks were excised from the midrib of a young leaf on an actively growing horseweed plant. Five disks from an individual plant were placed in a 50-ml clear-glass vial containing 1 ml of 10 mM $NH_4H_2PO_4$[1] plus 0.1% v/v Tween,[2] made up in deionized water (solution A) or in solution B, made up of solution A plus glyphosate[3] at concentrations of 1.3, 2.6, 5.3, 10.6, 21.2, and 42.3 mg ae L^{-1}. The vials were capped and placed horizontally on a tray covered in reflective foil and incubated under supplemental light (200 μmol m^{-2} s^{-1}) at 22 C for 18 h. Following incubation, the vials were frozen at −20 C and then thawed in a 60 C forced-air oven. A 500-μl aliquot of 1.25 N HCl[4] was added to each vial, and samples were maintained at 60 C for an additional 15 min.

A 25-μl aliquot was withdrawn from the sample vial and added to a single 300-μl microtiter plate[5] well containing 100 μl of a 0.25% periodic acid[6] : 0.25% metaperiodate[7] solution. Microtiter plates were covered with clear lids and incubated at 22 C for 60 min to facilitate shikimic acid oxidation. After incubation, 100 μl of 0.6 mol L^{-1} sodium hydroxide[8] : 0.22 mol L^{-1} sodium sulfite[9] was added to each well, and the optical density at 380 nm was measured using a Dynex Technologies MRXII microplate reader[10] with Endpoint software. To minimize the effects of background absorbance, the data were corrected by subtracting the optical density of control wells containing incubation solutions and leaf disk extract from plants not exposed to glyphosate.

A shikimate standard curve was developed using known amounts of shikimic acid[11] in microtiter plate wells containing appropriate proportions of each solution used in the test wells. Using a new set of standard curves for each day the assays were conducted, the values of the optical density at 380 nm (OD_{380}) from all samples were corrected for background absorbance and converted to micrograms of shikimate accumulated per milliliter of solution during the incubation period.

Response of Known Glyphosate-Resistant (GR) and Glyphosate-Susceptible (GS) Biotypes. Two previously characterized (Shrestha et al. 2007) horseweed biotypes from Fresno, CA (36°47′58N, 119°57′16W) and Tulare County, CA (36°29′15N, 119°24′10W) were used to determine the response of known GR and GS biotypes to the in vivo shikimate assay. In February 2006 and March 2007, seed from each field collection was sown on the surface of commercial potting media[12] in 26 by 52 by 6 cm plastic trays in a greenhouse, and moist soil conditions were maintained with daily drip irrigation. After emergence, single seedlings were transferred into peat pellets[13] and grown to approximately a 5-cm rosette size in the greenhouse. After reaching sufficient size and being acclimated to outdoor temperatures, seedlings were transplanted in March 2006 and April 2007 into 40-L pots filled with a 50 : 50 mixture of perlite and field soil (Hanford sandy loam). Plants were irrigated twice per day with a drip irrigation system and maintained throughout the summer growing season as a part of a related competition and water-stress experiment.

In June and July 2006 and August 2007, several experiments were conducted to determine the response of known GR and GS horseweed to in vivo glyphosate. The newest leaf of sufficient size was removed from seven randomly selected individual GR and GS horseweed plants at the bolting stage and five 4-mm leaf disks were excised from each leaf. Five of the resulting 35 leaf disks were randomly assigned to vials containing solution A (no glyphosate) or one of six glyphosate doses (1.3, 2.6, 5.3, 10.6, 21.2, and 42.3 mg ae L^{-1}) and assayed as previously described. Each treatment combination (biotype by glyphosate dose) was replicated three to four times in each experiment, and the data were analyzed as a completely randomized experiment.

Differences in mean shikimate accumulation between GR and GS horseweed biotypes were initially compared using 95% confidence intervals around the mean at each glyphosate dose in the 2006 experiments, and these results were used to determine a discriminating dose for the survey experiments. After all dose–response experiments were completed, the shikimate accumulation values were subjected to nonlinear regression[14] using a three parameter Gompertz model, assuming a normal distribution given by the following equation:

$$f = a \times \exp\{-\exp[-(x - I_{50})/b]\} \qquad [1]$$

Equation 1, where a is the upper response limit, x is the glyphosate concentration, I_{50} is the glyphosate rate that results in a 50% increase in shikimate accumulation, and b is a rate parameter related to the response to increasing glyphosate dose, was found to adequately fit data for both horseweed biotypes. The level of resistance was determined by calculating a resistant : susceptible (R : S ratio; I_{50} for the GR biotype divided by the I_{50} for the GS biotype).

Field Survey. A roadside horseweed survey was conducted during June through August of 2006 and 2007. Over the course of each growing season, several trips were made to collect horseweed leaf tissue from various areas in the Central Valley of California. Leaf tissue was collected from 61 sites in

seven counties in 2006 and from 84 additional sites from 12 counties in 2007, representing approximately a 100 by 400 km area. Because each sampling trip began and ended at the U.S. Department of Agriculture–Agricultural Research Service (USDA–ARS) San Joaquin Valley Agricultural Sciences Center, near Parlier, CA, a disproportionate number of samples were collected in the area between Fresno CA, and Visalia, CA. However, this area also appears to have one of the largest horseweed populations in the Central Valley (K. Hembree, personal communication).

The technicians collecting leaf material were instructed to drive along secondary roads and identify populations of horseweed in field margins, roadsides, canal banks, and noncrop areas. Numerous horseweed populations were identified in each year; however, no effort was made during the survey trips to record data on the presence or absence of horseweed between collection sites. At each collection site, several of the newest fully expanded leaves were collected from five individual plants that were bolting but had not yet flowered. Leaf disks were immediately cut from the leaves and placed into incubation solutions in the field during the 2006 survey; however, to increase efficiency in 2007, whole leaves were excised from plants, stored in vials of water, and brought back to the laboratory for processing. Fifteen leaf disks were extracted from three or four leaves per individual horseweed plant, and five disks were randomly assigned to incubation solutions containing 0, 5.3, and 42.3 mg ae L^{-1} of glyphosate. The remainder of the assay and the shikimate data transformations were conducted as described previously. In at least one assay each week, leaf tissue from outdoor-grown GR and GS biotypes at a similar growth stage was included to verify the assay procedures and control for environmental and growth stage effects.

Individual plants assayed in the survey experiment were scored as resistant, intermediate, or susceptible based on the 95% confidence limits around the mean shikimate accumulation of known GS and GR horseweed plants exposed to 5.3 mg ae L^{-1} in vivo glyphosate. If a plant accumulated less than 12.1 μg of shikimate L^{-1}, it was classified as resistant, whereas if it accumulated more than 20.5 mg L^{-1} of shikimate, it was classified as susceptible to glyphosate. Plants that accumulated between 12.1 and 20.5 mg L^{-1} of shikimate were classified as intermediate in response to glyphosate.

Simple geospatial analyses were conducted to test for regional and cropping system effects on the presence of the GR horseweed biotype. The coordinates and glyphosate response proportions from each collection location were entered into a geographical information system (GIS), and county boundaries and land-use data layers were superimposed using ArcMap.[15] To determine whether there were regional differences in the presence of GR horseweed in the Central Valley, individual sites were grouped into six regions, which each contained samples from one to five counties and were grouped from north to south: (1) Sacramento metro, which included Placer, Solano, Sutter, Yuba, and Yolo counties; (2) San Joaquin/Stanislaus counties; (3) Merced/Madera counties; (4) Fresno County; (5) Tulare County; and (6) Kings/Kern counties.

In an attempt to correlate the presence of GR horseweed to cropping systems, the proportion of GR, glyphosate-intermediate, and GS plants at each collection site was compared with land-use class. Land-use class and subclass within 5, 25, and 100 m of each collection site was determined from field collection notes and publicly available land-use data (CADWR 2008). Because of small sample size for individual subclasses, land-use data were grouped into five broad land use categories: (1) annual crop, (2) perennial crop, (3) pasture, (4) native vegetation, and (5) urban. The annual crop category included cereal grains, rice (*Oryza sativa* L.), cotton, corn, silage crops, and truck nurseries and berry crops, such as blackberry (*Rubus* spp.), melons (*Cucumis* spp., *Citrullus* spp.), tomatoes (*Solanum lycopersicum* L.), flowers, and perennial crop nurseries. Perennial crops included deciduous fruits and nuts, such as peaches [*Prunus persica* (L.) Batsch], European plums (*Prunus domestica* L.), nectarine [*Prunus persica* Batsch var. *nectarina* (Aiton) Maxim.], almonds, walnuts, and pistachios, all citrus (*Citrus* spp. L.), and all grapes (wine, table, and raisin). Pasture included alfalfa (*Medicago sativa* L.) and alfalfa mixes, clover (*Trifolium* spp. L.), mixed pasture, and native pasture. Native vegetation included lands classified as grasslands, brush, timber, and forest, as well as surface waters and barren lands. The urban land class included residential, commercial, industrial, urban landscape, paved and unpaved roads, and railroad rights of way. Semiagricultural lands, including farmsteads, feedlots, and dairies, were included in the urban class for these analyses.

The main effects of region and cropping system on proportion of GR individuals in a population were analyzed using ANOVA,[16] and means were separated using Fisher's Protected LSD with $\alpha = 0.05$.

Results and Discussion

Response of Known GR and GS Biotypes. Plants from the horseweed biotype previously shown to be susceptible to glyphosate on the whole-plant level (Shrestha et al. 2007) accumulated more shikimate than the known resistant biotype at all in vivo glyphosate concentrations between 1.3 and 21.2 mg ae L^{-1} (Figure 1). The shikimate accumulation by the GS biotype was greater than the no-glyphosate control treatments in all glyphosate concentrations tested, whereas the GR biotype required at least 10.3 mg ae L^{-1} to accumulate statistically more shikimate than the control. Although the GR biotype accumulated little shikimate at lower glyphosate doses, at concentrations of 21.2 mg ae L^{-1} or higher, shikimate was accumulated at a rate similar to the GS biotype. The nonlinear regression was highly significant for both horseweed biotypes, and coefficient estimates were significantly different among horseweed biotypes for all three parameters (Table 1). The predicted upper response limit was almost 60% higher for the GS biotype compared with the GR biotype, although the actual mean values were not statistically different. Similarly, the *b* rate parameter differed among horseweed biotypes with a much slower rate of increase in the GR biotype for a given increase in glyphosate concentration. Comparison of the I_{50} estimates from the in vivo assay indicates that this GR horseweed biotype has a 4.8-fold level of resistance.

This level of resistance is consistent with the sixfold level of whole-plant resistance reported by Shrestha et al. (2007) with the same biotypes and is similar to R : S ratios of 4 to 12 reported in other North American horseweed biotypes exposed to foliar glyphosate treatments (Main et al. 2004; Van Gessel 2001). Although the absolute amount of

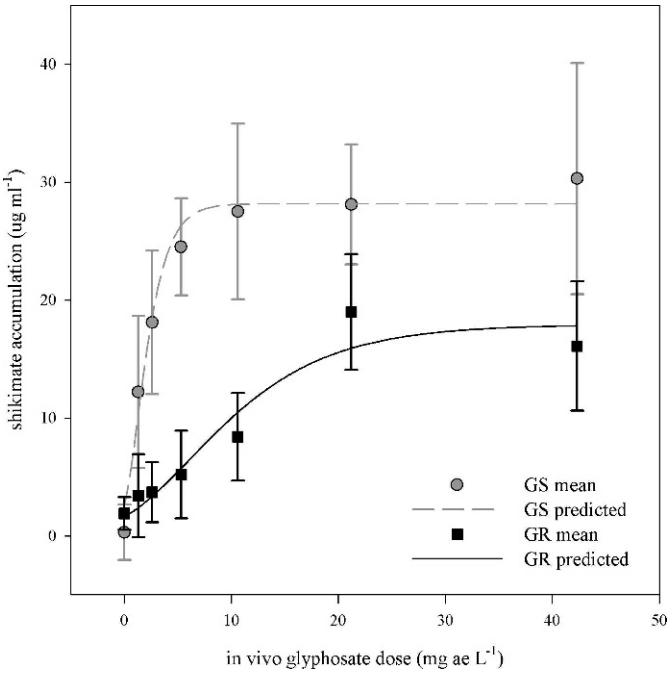

Figure 1. Shikimate accumulation in known glyphosate-resistant (GR) and glyphosate-susceptible (GS) horseweed biotypes in response to in vivo glyphosate. Symbols and error bars are the mean value and the 95% confidence intervals for shikimate accumulation at each glyphosate concentration, and lines are predicted values based on a three-parameter Gompertz function.

shikimate accumulated in these horseweed biotypes was similar to those reported by Koger et al. (2005), our results were slightly more variable most likely because of the purity of the seed collection and overall plant health of the assay plants grown under high heat and low relative humidity summer conditions common in the Central Valley.

Initially, a ratio of shikimate accumulation between the 5.3 and 42.3 mg ae L^{-1} glyphosate concentrations appeared promising for discriminating between GR and GS plants (data not shown). However, based on the results of preliminary and complete in vivo dose–response experiments conducted in 2006 (not shown and included in Figure 1), an in vivo glyphosate concentration of 5.3 mg ae L^{-1} provided the most accurate and robust classification of the test biotypes grown outdoors under summer conditions in the Central Valley. Using this dose, the known GR and GS plants would have been correctly identified 88 and 95% of the time, respectively (data not shown) during the course of the experiments. These methods provided consistent results on the test biotypes from growth stages between small rosette to late bolting as long as tissue from the youngest leaves was used (data not shown). Thus, 5.3 mg ae L^{-1} glyphosate was used for final discrimination between GR and GS horseweed plants in the field survey experiments.

Table 1. Model parameter estimates, standard errors, and P values estimated by nonlinear regression for shikimate accumulation by known glyphosate-susceptible (GS) and glyphosate-resistant (GR) horseweed biotypes from the Central Valley of California as affected by in vivo glyphosate dose.

Population	Model adjusted R^2	Parameter[a]	Estimate	Standard error	P value
GS	0.84	*a*	28.2	1.7	< 0.0001
		b	1.5	0.5	0.0062
		I_{50}	1.3	0.4	0.0004
GR	0.74	*a*	17.9	1.9	< 0.0001
		b	7.0	2.3	0.0035
		I_{50}	6.2	1.6	0.0002

[a] Parameter abbreviations: *a*, upper response limit; *b*, slope at I_{50}; I_{50}, glyphosate concentration required to increase shikimate accumulation by 50%.

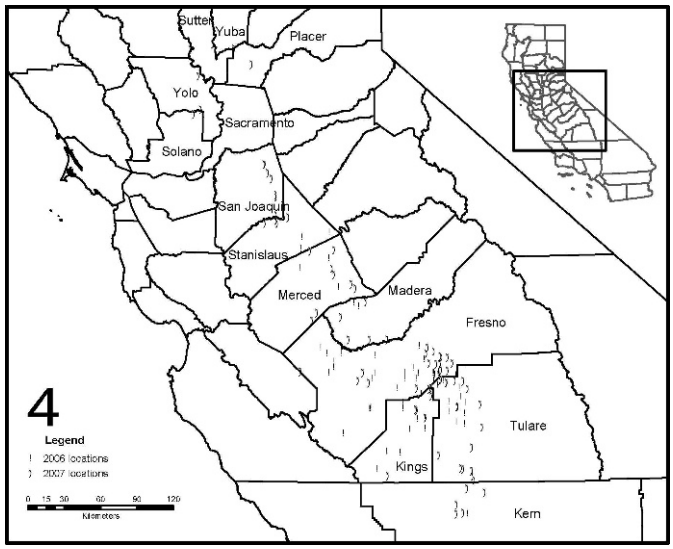

Figure 2. Horseweed tissue collection locations in a glyphosate-resistant horseweed roadside survey conducted in the Central Valley of California during 2006 to 2007.

Field Survey. Leaf tissue collected from a total of 305 individual plants at 61 locations in 2006 and 415 individuals collected at 83 locations in 2007 was subjected to the in vivo shikimate assay using 5.3 mg ae L^{-1} glyphosate as the test concentration. Data from several individuals were removed each year before final analysis because of out-of-normal-range results (usually negative-corrected OD values) assumed to be due to either poor leaf tissue condition at collection or a pipetting error during the assay reactions; however, a total of 574 individuals from 141 locations were included in the final analysis (Figure 2). Overall, 23% of the individuals sampled in 2006 and 2007 were classified as GS, 62% were GR, and 15% were intermediate in response to the in vivo shikimate accumulation assay. Although it was somewhat surprising that the majority of the individual plants were resistant to glyphosate, this result likely was affected by the timing of the survey. Most of the leaf tissue samples were collected in summer, after the horseweed plants were bolting and were easily identified from a moving vehicle; thus many GS plants may have already been removed from the population with spring glyphosate applications. Although the proportion of GR individuals may have been inflated by the survey methods, it is clear that GR horseweed is widely distributed in the Central Valley of California. In direct contrast to the GR individuals, the proportion of GS individuals may have been underestimated by this summer survey, but it appears that most locations have a mixed population of resistant, intermediate, and susceptible horseweed. It is not known whether the intermediate individuals identified in this survey expressed an intermediate phenotype because of heterozygosity, intermediate enzyme activity or gene expression, or simply as an artifact of the collection and assay procedure.

A main effects analysis was conducted to determine whether GR horseweed was associated with geographic areas in the Central Valley or with primary land use near the collection

Table 2. Main effects analysis of region and land use class on the presence of glyphosate-resistant horseweed in the Central Valley of California in a 2006 to 2007 roadside survey.

Region and class	n[a]	Resistant	Intermediate	Susceptible
		%		
Central Valley region				
Sacramento metro counties[b]	6	20	25	55
San Joaquin/Stanislaus counties	15	69	16	15
Merced/Madera counties	23	64	21	15
Fresno County	47	69	12	19
Tulare County	28	65	13	22
Kings/Kern counties	21	49	14	37
$LSD_{(0.05)}$	—	26	NS	20
Land use class				
Annual crop	33	65	13	23
Perennial crop	57	60	13	27
Pasture	12	70	19	10
Native veg. / noncrop	16	58	22	20
Developed	22	62	17	22
$LSD_{(0.05)}$	—	NS	NS	NS

[a] Number of collection sites in each region or land-use class. Each site includes data from five individual horseweed plants in the population.

[b] The Sacramento metro counties include Placer, Solano, Sutter, Yolo, and Yuba counties.

site. Overall, region and primary land use had little, or no, effect on the proportion of resistant, intermediate, and susceptible horseweed in this survey (Table 2). On a regional basis, the Sacramento metro counties, the northern-most area in this survey, had fewer GR individuals and had more GS individuals than most other regions. The southern-most region, Kings and Kern counties, had an intermediate number of GS individuals compared with the other regions. Sacramento metro samples, which were collected from three urban, one annual crop, one pasture, and one native vegetation site, tended to have fewer GR and more GS individuals; however, the small sample size for this region ($n = 6$) minimizes the strength of any interpretation.

Contrary to our originally hypothesis, land-use class was not correlated with the proportion of glyphosate-resistant, glyphosate-intermediate, or glyphosate-susceptible horseweed in the Central Valley (Table 2). Resistant plants made up 58 to 70% of the population, whereas GS plants were 10 to 27% of the population across all land-use classes. The methods used in this survey combined with the biology of horseweed provide a likely explanation for these results. The leaf tissue used in the assays for the survey was usually collected from horseweed plants growing in field margins, roadsides, canal banks, and unmanaged areas near cropped and urbanized areas. The weed control strategies used in these areas may have been different with respect to glyphosate than those used within the production fields and may have provided different selection pressure for the GR biotype regardless of nearby crops. More important, perhaps, horseweed is a highly prolific seed producer, and the wind-disseminated seed can quickly spread the species over a wide area (Dauer et al. 2007). It is very likely that many of the GR individuals were not selected in the field in which they were sampled; thus, the nearby crops may have had little effect of the presence or proportion of GR horseweed at a given location.

In retrospect, an important flaw was determined with this field survey (Beckie et al. 2000). The methods used in this survey, while relatively efficient, unfortunately did not allow analysis of the level of total horseweed infestation in the Central Valley. For example, although several survey trips were made in western Kern, Kings, Fresno, and Merced counties, few populations were identified and sampled (Figure 2). Field notes and anecdotal evidence suggest that horseweed only sparsely infests this part of the Central Valley, which is characterized by annual crops, including cotton, tomato, and vegetables, as well as frequently disturbed crops such as alfalfa. Similarly, relatively few samples were collected in eastern Tulare and Fresno counties, which are primarily devoted to citrus production; these cropping systems generally use various strategies to keep orchards free of weeds to reduce frost risks. Conversely, in eastern Tulare and central Fresno counties, horseweed is very commonly found in and around the main orchard and vineyard crops. Thus, although the proportion of GR and GS horseweed did not differ among cropping systems, it is likely that the total population would have been affected by either cropping system or region had those data been collected.

The GR biotype had a 4.8-fold level of resistance in these studies based on the in vivo shikimate assay and a sixfold level based on the whole-plant studies of Shrestha et al. (2007). This level of resistance suggests that the mechanism of resistance likely is reduced glyphosate translocation, as observed in several *Conyza* and *Lolium* species (Dinelli et al. 2008; Feng et al. 2004; Koger and Reddy, 2005; Perez-Jones et al. 2007; Shaner 2008; Wakelin et al. 2004), rather than an altered target site. Although the level of resistance observed in GR plants is relatively low, glyphosate rates high enough to control this biotype are not likely to be economically feasible. Furthermore, previous research with the same biotypes suggests that, at later growth stages, glyphosate tolerance of both GR and GS horseweed increases (Shrestha et al. 2007), which would suggest that multiple high-application rates would be required in these cropping systems. The results of the GR horseweed survey indicate that the resistant biotypes are already widely distributed in the Central Valley. Most locations tested had a majority of resistant and intermediate individuals in the population, regardless of region or nearby annual and perennial crops or noncrop areas. This is similar to the results of a recent multiyear survey in Indiana, where widespread GR horseweed was found in a soybean-based cropping system due to the adoption of glyphosate-tolerant crops (Davis et al. 2008).

GR horseweed is a widespread, economically important weed problem in this diverse and high-value cropping region in California. Previous research in the state indicated that this GR horseweed biotype is more vigorous than GS horseweed under similar conditions (Grantz et al. 2008; Shrestha et al. 2007; B. D. Hanson, unpublished data), which suggests that the problem is likely to continue or worsen. A related weed, hairy fleabane, with a similar level of resistance to glyphosate has been recently reported in California (Shrestha et al. 2008), further emphasizing that glyphosate-based weed-control strategies must be replaced with integrated strategies, using both cultural practices and other herbicides to provide control of the weed spectrum in these cropping systems.

Sources of Materials

[1] Ammonium phosphate monobasic, American Chemical Society (A.C.S.) grade, Fisher Scientific, 1 Liberty Lane E, Hampton, NH 03842.

[2] Tween 20, Aldrich Chemical Co. P.O. Box 355, Milwaukee, WI 53201.

[3] Roundup Ultramax, isopropylamine salt of glyphosate, Monsanto Co., 800 North Lindbergh Boulevard, St. Louis, MO 63167.

[4] Hydrochloric acid 37%, A.C.S. grade, Mallinckrodt Chemical Works, 2nd and Mallinckrodt Street, St Louis, MO 63167.

[5] Fisherbrand flat bottom 96 well plate, Fisher Scientific, 1 Liberty Lane E, Hampton, NH 03842.

[6] Periodic acid, Aldrich Chemical Co., P.O. Box 355, Milwaukee, WI 53201.

[7] *m*-periodate, Aldrich Chemical Co., P.O. Box 355, Milwaukee, WI 53201.

[8] Sodium hydroxide, A.C.S. grade, Fisher Scientific, 1 Liberty Lane E, Hampton, NH 03842.

[9] Sodium sulfite anhydrous, A.C.S. grade, Mallinckrodt Chemical Works, 2nd and Mallinckrodt Street, St Louis, MO 63167.

[10] MRX II microplate absorbance reader, Dynex Technologies Inc., 14340 Sullyfield Circle, Chantilly, VA 20151.

[11] Shikimic acid, Technical grade, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178.

[12] Metro-Mix 200 potting mix, Sun Gro Horticulture, Inc., 15831 NE 8th Street, Suite 100, Bellevue, WA 98008.

[13] Jiffy-7 peat pellets, Jiffy Products of America Inc., 600 Industrial Parkway, Norwalk, OH 44587.

[14] SigmaPlot for Windows, Version 10.0, 2006, Systat Software Inc. 225 W, Washington Street, #425, Chicago, IL 60606.

[15] ArcMap Version 9.2, 2006, ESRI Inc., 380 New York Street, Redlands, CA 92373-8100.

[16] SAS software, Version 9.1, 2003, SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC 27513.

Acknowledgments

Technical assistance for this research was received from Ms. Stella Zambrzuski, Ms. Karen Pelham, Ms. Idalia Orellana, and Ms. Ashley Torres. Partial funding for student interns was received from the Louis Stokes Alliance for Minority Participation Program, the USDA-ARS Pacific West Area Summer Intern Program, and from the Garden Rose Council. This article is a U.S. government work and is in the public domain in the United States. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

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Received June 19, 2008, and approved September 26, 2008.